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The Connection between Urinary Equol Levels and the Prevalence of Atopic Dermatitis

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Keywords
Soy isoflavone · Equol · Daidzein · Genistein · Atopic dermatitis

Abstract
Background: Soy isoflavones and their metabolites such as equol have been associated with a reduced risk of hormone-sensitive tumors and metabolic syndromes. However, individual soy isoflavones and equol levels in atopic dermatitis remain uninvestigated. ***Objective:*** The aim of this study is to compare the levels of urinary daidzein, genistein, and equol between atopic dermatitis patients and normal subjects and to examine the correlation between equol concentration and the severity of clinical symptoms. ***Methods:*** A cross-sectional study was conducted at Akita University Hospital and Aso Iizuka Hospital in Japan. Fifty patients with confirmed atopic dermatitis diagnosis and 67 healthy controls were recruited. Daidzein, genistein, and equol in urine were measured by using a high-performance liquid chromatography-mass spectrometry system. ***Results:*** Urinary equol levels were significantly lower in the atopic dermatitis patients than in the healthy controls ($p = 0.002$). The difference was particularly noticeable in young people (6–19 years, $p < 0.001$). No correlations were found between urinary equol levels and the severity of clinical symptoms and laboratory data in the atopic dermatitis patients. ***Conclusion:*** Equol levels in childhood might be involved in the development of atopic dermatitis.

Introduction

Isoflavones contained in soy protein have been investigated for their health benefits [1–3]. In humans, daidzin and genistin, which are major isoflavones in soy protein, are converted to daidzein and genistein by β-glucosidase. Daidzein is metabolized to dihydrodaidzein and then converted to equol ((3S)-3-(4-hydroxyphenyl)-3,4-dihydro-2*H*-chromen-7-ol) or O-DMA by specific intesti-

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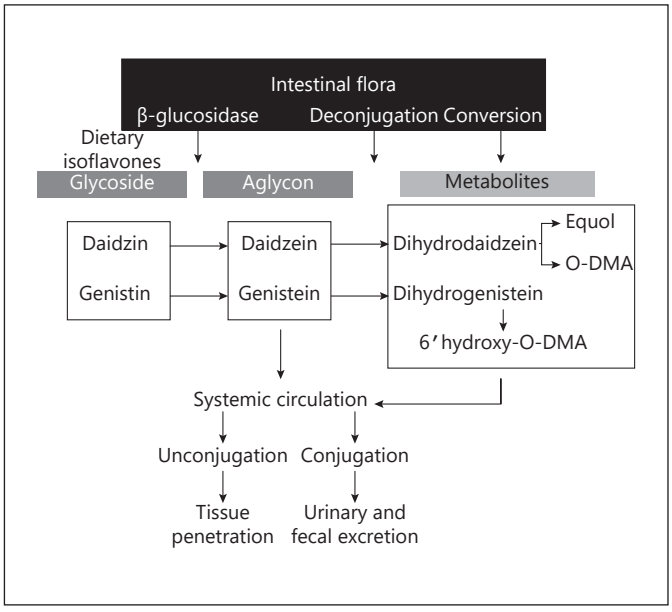

Fig. 1. Metabolism of daidzin to equol in the intestine. O-DMA, O-desmethylangolensin.

nal bacteria [4, 5] (Fig. 1). However, the prevalence of equol, which means the existence of equol-producing flora, was reported about 25% among adults on a regular diet in China [6]. The beneficial effects of equol have been the subject of particular focus because it was reported that equol possesses strong estrogen-like, anti-androgen, and anti-oxidative activity [7–9]. Thus, equol producers are known to have preventive effects for osteoporosis, metabolic syndrome, breast cancer, or prostate cancer compared with nonproducers [10–12]. Equol supplementation was also shown to reduce aging wrinkles in postmenopausal women [13].

Atopic dermatitis (AD) is a chronic and relapsing eczematous disease generally associated with allergen-induced skin inflammation. Chronic persistent inflammation due to both free radical and inappropriate T helper (Th) cell activation by estrogen decrease is postulated to impair the skin barrier function and to modulate the immunological profile of AD [14, 15]. Clinically, the exacerbation of cutaneous symptoms in AD is known to be associated with menstruation, when estrogen declines [16]. Therefore, excessive oxidative stress and decreases in estrogen levels are considered to exacerbate the inflammatory process or to be involved in the development of AD. From this evidence, we hypothesized that soy protein-derived isoflavones and equol might be involved in the development of AD or the severity of its symptoms.

In the present study, we first measured the levels of urinary daidzein, genistein, and equol in AD patients and compared them with those in normal subjects. In addition, we investigated the correlation between urinary equol levels and the severity of skin symptoms and laboratory data in AD patients.

Materials and Methods

Participants and Design

Fifty patients from Akita University Hospital and Aso Iizuka Hospital (mean age: 28.5 years; age range: 6–77 years; 18 females, 32 males) with AD diagnosed in accordance with the criteria of the AD clinical guidelines from the American Academy of Dermatology [17] were registered with the study after their written informed consent was obtained. Each patient was examined for white blood cell count (WBC), immunoglobulin E (IgE), peripheral eosinophil count, thymus and activation-regulated chemokine (TARC), and lactate dehydrogenase (LDH), and the severity of the eczema was evaluated based on the Eczema Area and Severity Index (EASI). The patients' detailed clinical and laboratory data are summarized in Table 1. Patients who were undergoing systemic immunosuppressive or antihistamine therapy were included. Sixty-seven Akita University Hospital staff members or their children (mean age: 35.4 years; 31 females, 36 males) who had no skin disease were also included as the healthy control group, from whom informed consent was also obtained. Upon obtaining informed consent, we asked all the participants to fill out a habitual weekly soy food intake questionnaire and we calculated the intake of soy protein using the database of the National Institute of Health and Nutrition, Japan. The first-void urine of the morning was collected and stored at −80°C in a freezer until the isoflavone metabolites were measured (Urinary isoflavones are known to be stable at room temperature for 14 days [18]). This research was approved by the ethics committees of the Akita University Graduate School of Medicine and Aso Iizuka Hospital.

Measurement of Isoflavones and Metabolites in Urine

Daidzein, genistein, and equol in urine were measured by using a high-performance liquid chromatography-mass spectrometry system. These measurements were performed by LSI Medience Corporation (Tokyo, Japan). The accuracy, reproducibility, and quantitativeness of this method are verified by the company. It has been known that the concentrations of urinary daidzein, genistein, and equol correspond to those of serum daidzein, genistein, and equol, respectively [19].

Statistical Analyses

All analyses were carried out using BellCurve for Excel Version 2.00 (Social Survey Research Information Co., Ltd., Tokyo, Japan). Data were represented as mean and standard deviation (SD). Student's *t* test was used to determine the significance of differences between the 2 groups. The correlations among the clinical and laboratory data in AD patients were assessed by calculating Spearman's rank correlation. R and R^2 revealed the correlation coefficient and the coefficient of determination, respectively. *p* values were two-sided; those under 0.05 were considered to be statistically significant.

Table 1. The characteristics of the cohort in the present study

Variable	AD patients	Healthy control	*p* value
Subjects, *n*	50	60	
Age, years, mean±SD	28.5±14.3	35.4±18.4	0.98
Sex, M:F, *n*	32:18	31:36	0.08
Soy protein intake, g/week, mean±SD	33.6±20.2	31.3±17.3	0.532
Serum IgE, IU/mL, mean±SD	7,343.3±13,480.6	N/A	
Eosinophils, %, mean±SD	6.9±6.1	N/A	
TARC, pg/mL, mean±SD	3,850.9±13,432.4	N/A	
LDH, U/L, mean±SD	254.6±104.2	N/A	
EASI score, mean±SD	12.0±11.2	N/A	
Treatment, *n*			
Emollient	50	N/A	
Topical tacrolimus	15	N/A	
Topical steroid	40	N/A	
Anti-histamine	18	N/A	
Cyclosporine	6	N/A	

IgE, immunoglobulin E (normal range: <170 IU/mL); TARC, thymus and activation-regulated chemokine (normal range: <450 pg/mL); LDH, lactate dehydrogenase (normal range: 200–400 U/L); EASI, Eczema Area and Severity Index. *p* values under 0.05 are considered statistically significant.

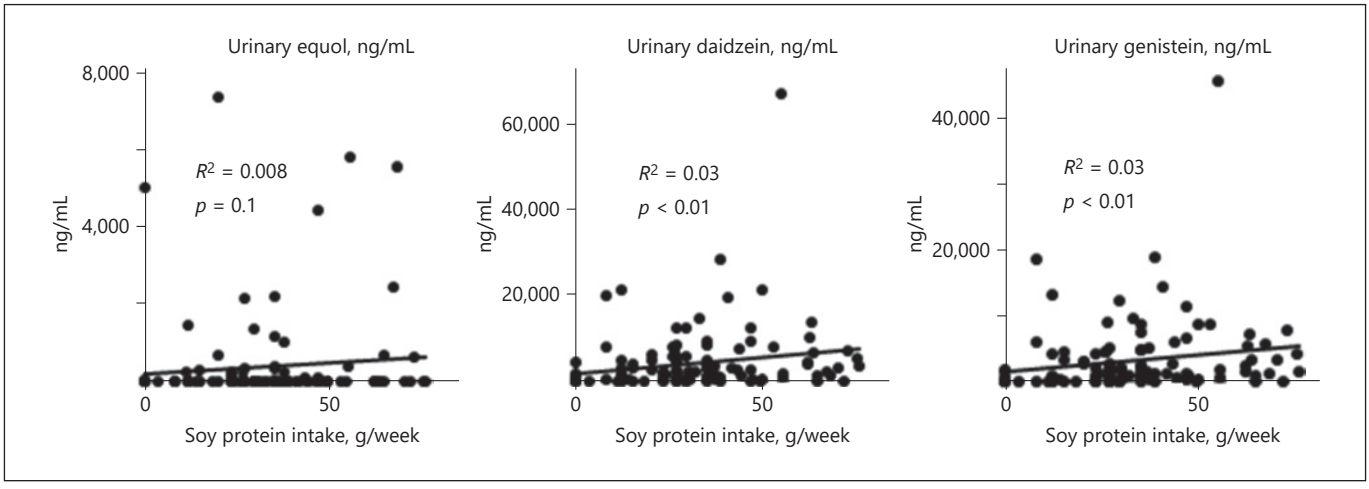

Fig. 2. Correlations between urinary equol, daidzein, and genistein and soy protein intake. The levels of urinary daidzein and genistein show significant correlations for soy protein intake. No correlation is seen for equol levels. *p* values under 0.05 are considered statistically significant.

Results

Baseline Clinical Characteristics

The clinical and laboratory data of the AD patients and the control subjects are given in Table 1. There was no significant difference in age, sex, or soy protein intake between the 2 groups. In the AD patients, the serum IgE, TARC, and LDH, which are known to be atopic inflammatory markers, were higher than the standard values. The mean EASI score in the AD patients was 12.0 ± 11.2. The majority of patients were treated with emollient and topical steroid. Thirty six percent and 12% of the AD patients were prescribed oral anti-histamine and cyclosporine, respectively.

Soy Protein Intake and Urinary Soy Isoflavones

As shown in Figure 2, the levels of urinary daidzein and genistein, which are soy isoflavone metabolites of

Table 2. The levels of urinary soy isoflavones and equol in the AD patients and in the healthy controls

Variable	AD patients (n = 50)	Healthy controls (n = 67)	p value
Daidzein, ng/mL, mean±SD	5,591.5±10,721.8	3,562.0±4,485.9	0.93
Genistein, ng/mL, mean±SD	4,054.0±7,527.5	2,447.3±2,934.5	0.58
Equol, ng/mL, mean±SD	389.7±1,312.5	390±1,136.8	0.002

AD, atopic dermatitis. p values under 0.05 are considered statistically significant.

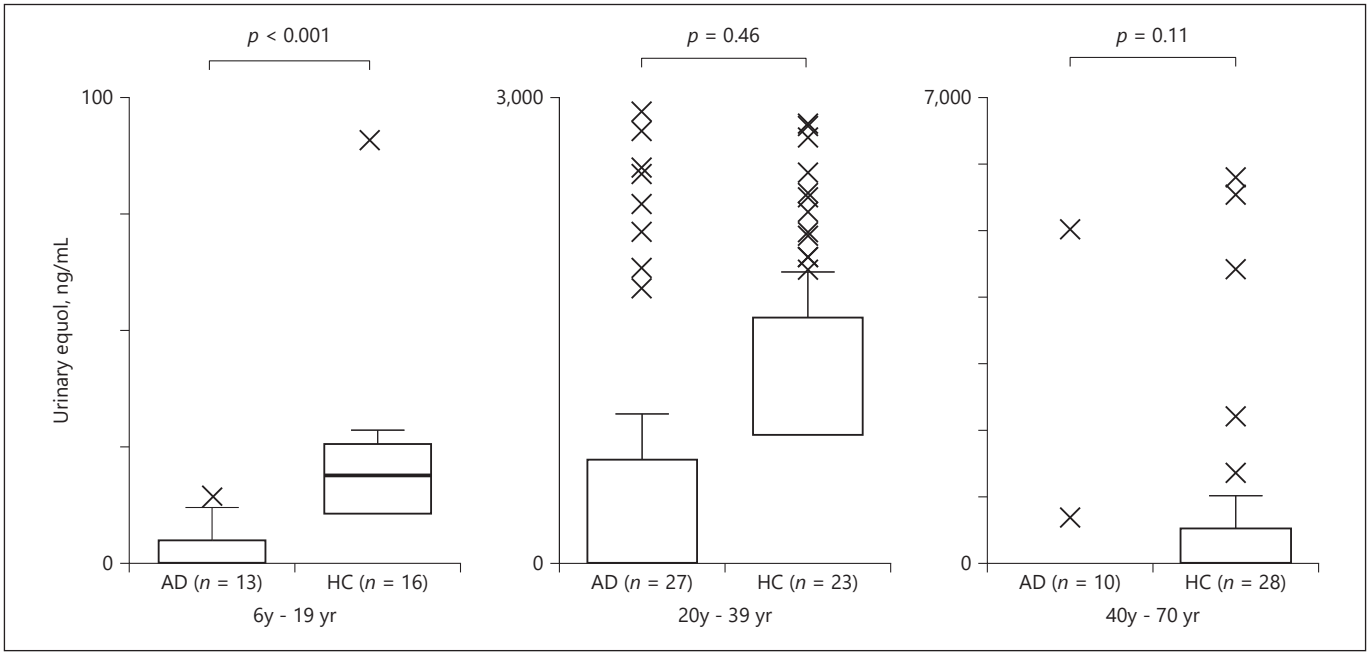

Fig. 3. Urinary equol levels in patients with AD and in HC by age. In subjects aged 6–19 years, the equol levels are significantly lower in the AD patients than in the HC. In 77% of the AD patients younger than 19 years, equol was below the detection limit. p values under 0.05 are considered statistically significant. AD, atopic dermatitis; HC, healthy controls.

β-glucosidase, correlate positively with soy protein intake in the trial subjects (r^2 = 0.03, p < 0.01, r^2 = 0.03, p < 0.01). This result is consistent with a previous report that showed most people to have metabolic activity involving β-glucosidase [20]. In contrast, there was no correlation between urinary equol levels and soy protein intake. The difference is attributed to whether the subject harbors equol-producing bacteria or does not.

Urinary Equol Levels in the AD Patients

The urinary equol levels were significantly lower in the AD patients than in the healthy controls; however, there were no significant differences in urinary daidzein and genistein levels (Table 2). Next, age is known to be involved in equol prevalence [21], so we compared urinary equol levels by age. Notably, urinary equol levels in patients with AD (ages 6–19) were found to be significantly lower than those in the healthy control group (Fig. 3). Most of the patients were below the detection limit.

Association among AD-Related Laboratory Data and Urinary Equol in the AD Patients

In the AD patients, the elevation of serum IgE levels and the peripheral blood eosinophilia that were associated with atopy and with increases in TARC and LDH were also found to be associated with skin inflammation. Thus, we calculated the correlation coefficients among urinary equol, laboratory data, and severity of skin symp-

Table 3. Correlations between urinary equol level, laboratory data, and EASI score in AD patients

	WBC, μ/L	Eosinophills, %	LDH, U/L	TARC, pg/mL	IgE, IU/mL	EASI	Equol, ng/mL
(a) Correlation coefficients for each clinical and laboratory data, including urinary equol level and EASI score							
WBC	1.0000	0.3970	0.6358	0.5470	0.5002	0.2557	−0.2452
Eosinophills	0.3970	1.0000	0.7397	0.7301	0.2020	0.0617	−0.0966
LDH	0.6358	0.7397	1.0000	0.8924	0.4894	0.2391	−0.0708
TARC	0.5470	0.7301	0.8924	1.0000	0.5569	0.1900	−0.0636
IgE	0.5002	0.2020	0.4894	0.5569	1.0000	0.4068	−0.0421
EASI	0.2557	0.0617	0.2391	0.1900	0.4068	1.0000	0.0857
Equol	−0.2452	−0.0966	−0.0708	−0.0636	−0.0421	0.0857	1.0000
(b) Statistical differences for each clinical and laboratory data, including urinary equol level and EASI score							
WBC	–	0.0043	$p < 0.001$	$p < 0.001$	$p < 0.001$	0.731	0.862
Eosinophills		–	$p < 0.001$	$p < 0.001$	0.1596	0.6704	0.5046
LDH			–	$p < 0.001$	$p < 0.001$	0.945	0.6250
TARC				–	$p < 0.001$	0.1864	0.6609
IgE					–	0.00034	0.7714
EASI						–	0.5541
Equol							–

The correlation coefficients were calculated by Spearman's rank correlation coefficient. AD, atopic dermatitis; IgE, immunoglobulin E; TARC, thymus and activation-regulated chemokine; LDH, lactate dehydrogenase; EASI, Eczema Area and Severity Index. Statistical differences were calculated by Fisher's Z-transform.

toms using the EASI score (Table 3). Levels of LDH, TARC, and IgE tended to correlate positively with the EASI score, as previously reported [22]. However, no tendency was found for laboratory data to correlate with urinary equol. These results suggest that equol levels might not be associated with the severity of the skin manifestations or with the allergic condition.

Discussion

Dysbiosis of the intestinal flora has been reported to be associated with various diseases beyond just inflammatory bowel disease [23, 24]. An investigation of intestinal flora found counts of aerobic bacteria such as coliforms and *Staphylococcus aureus* to be higher in 2-year-old children with allergic diseases such as bronchial asthma and allergic rhinitis than in children without allergic diseases [25]. This association is also found in skin allergic diseases including AD. A large study controversially demonstrated that higher prevalence of *Clostridia* in the intestinal flora is associated with increased risk of new-onset AD [26]. Notably, the supplementation of bacteria of the *Lactobacillus acidophilus* L-92 strain significantly improves skin symptoms in infants with AD [27]. These reports indicate that probiotics or intestinal flora transformation might be a potential therapeutic approach for AD.

In this study, we focused on equol, which is a daidzein metabolite that is produced by specific intestinal flora and that has a strong estrogen-like effect [1] (Fig. 1). Daidzein, which is converted to equol, is an isoflavone that abounds in soybeans. Equol's estrogen-like effect is believed to reduce the incidence and mortality of breast cancer, prostate cancer, and heart disease [5, 7, 8]. In the present study, we revealed the urinary equol concentration to be significantly lower in AD patients than in healthy controls. This result means that equol might be related to the AD pathogenesis. As it was reported that male AD patients have lower levels of estradiol than healthy controls [15], the immune responses of AD patients could be influenced by sex hormones. Furthermore, estrogen and progesterone enhance the activities of Th2/regulatory T cells but suppress Th1/Th17 [28]. The skin permeability barrier is reinforced by estrogen but impaired by progesterone and androgens [28]. Thus, the estrogen-like effect of equol may improve AD symptoms in tandem with an anti-oxidative effect.

The prevalence of AD has been increasing in Asia in recent years. This has been attributed to the Westernization of lifestyle and diet in Asia [29]. On the other hand, soy protein intake is known to be higher in Asians than

in Europeans and Americans due to Asian dietary culture. Intriguingly, equol production from soy protein depends on individual intestinal flora, and the composition of flora is different in each country [6]. In parallel with soy consumption, 50% of Japan's population can produce equol, versus only about 30% of the population in Western countries [13]. We noticed that the number of AD patients used to be high in Western countries but low in Asian countries including Japan, whereas the intake of soy protein necessary for equol production has been higher in Asian countries than in Western countries [30]. The difference in soy protein consumption differed by a factor of 100 between Americans and Japanese in 2007 (http://www.fao.org/faostat/en/#home, Accessed June 1, 2020). Taking the above together, we hypothesize that areas with higher soybean consumption or equol prevalence will be those with milder AD. Although we found no correlation between urinary equol level and skin manifestation severity (Table 3), we did find that few equol was detected in pediatric AD patients (Fig. 3). Thus, our results suggest that equol production by pediatric intestinal flora from soy protein ingestion may be a preventive factor for the development of AD. Naturally, the number of patients in this initial study is limited; however, this is the first study to elucidate equol concentration in AD patients. The equol level was lower in the AD patients than in the normal subjects. Moreover, the prevalence of equol in childhood may be related to the onset of AD. Further studies are warranted toward exploring whether equol supplements might prevent the onset of AD.

Statement of Ethics

The study design was approved by the ethics committees of the Akita University and Aso Iizuka Hospital. This study was conducted in accordance with the study protocol and the Declaration of Helsinki. Patients willing to participate in this study were asked to provide their written informed consent after being given sufficient time to consider participating.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

Funding Sources

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Author Contributions

T.C., F.K., and T.N. collected the data. T.C. and T.N. analyzed the data. M.K. contributed to the interpretation of the data. All authors contributed to the design and draft of the work and read and approved the final manuscript.

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